EXPLANATION OF FIGURES.

FIG. I. Front view of reservoir showing straps and attachments, pump, handle, lance, and hose.

II. Back view of reservoir showing pump, handle, lance, hose, and fulcrum; also manner of attaching the latter.

III. Top view of reservoir. Top view of pump, opening 2½ inches in diameter *a*; opening for introduction of liquid, 7 inches long, 4½ inches wide, *b*; casting for holding the fulcrum, *c*; one-fourth actual size.

IV. Strainer, 7 inches long, 4½ inches wide, 1 inch deep; wire gauze soldered on the bottom, and handle *a* across the top; *b* and *c* lid, one-fourth actual size.

V. Pump complete. 1 one-fourth actual size; 2 and 3, one-half actual size. The plunger shown here has been abandoned and the one at Fig. VII substituted. The cross piece made of brass shown at *a* in 1 and 3 is retained in the new form. This piece holds the ball of the valve in place.

VI. Front view of reservoir showing pump inside; soldered at points seen at *a*.

VII. Plunger with ball valve showing ball at *a*, and space for packing at *bb*, actual size. The tube to which this is fastened is 14¾ inches long, making the total length with the piece marked *b*, in Fig. V, 17 inches.

VIII. Casting for attaching straps, *a*; fulcrum, *b*; casting which is soldered to reservoir *c*, as shown in Fig. II, and to which the lower end of the fulcrum is fastened by means of a bolt. All one-fourth actual size.

IX. Lance and nozzle one-fourth actual size.

X. Sprayer in use.

RECENT INVESTIGATIONS OF SMUT FUNGI AND SMUT DISEASES

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(*Continued.*)

As early as 1884 I began to make infection experiments on host plants, but soon, on account of the great number of details involved and the unimproved condition of my eyes, I was convinced that I could not carry through the experiments without assistance. Only upon my entrance into the Ministry of Public Instruction and transfer to Münster in Wesen was the help of an assistant in Mycology willingly granted me. This I had previously done without, having requested it of the Forest Department of the Ministry of Agriculture and been refused, although it was desired only as a compensation for my eye lost in direct government service. With this help, which I secured in the person of Dr. G. Istvanffi, privat docent in the University of Klausenburg in Hungary, I was able to bring the experiments to a relative conclusion.

But even four years ago I had carried on the culture of smut fungi in nutrient solutions for a long time, in order at least to make the first part of the investigations as complete as possible. I had cultivated the conidia of oat smut and corn smut from generation to generation for more than a year. Every four days the nutrient solutions were exhausted and the mass of yeast conidia was deposited in the culture as a distinct sediment. A few germs from the exhausted culture were always introduced by a needle-point into a new nutrient solution, and in another four days this was also exhausted. The serial cultures amounted to more than a hundred, which must have corresponded to about fifteen hundred continuous generations of the yeast conidia produced exclusively by sprouting. Yet the conidia produced in the last culture were of the same form as in the first. According to this, the sprout conidia in their unbroken succession are to be regarded as the exclusive product of the growth of these smut fungi in nutrient solutions *outside* of the host plants. This result is noteworthy in the same degree as the long-known fact that the smut spores are exclusively the form of the same smut fungi *inside* of the host plants.

Only this one change was to be observed in the continuous generations of the sprout conidia—they gradually pushed out into threads more slowly when the nutrient solution was exhausted. After ten months culture, after more than 1,000 sprout generations were formed, the germination in threads ceased entirely, the conidia swelled up somewhat and divided perhaps into two cells, but then remained passive. If we reflect that the conidia can penetrate into the host plants, to produce smut, only by means of their germ tubes, then with the disappearance of the tube germinations their infective power must also necessarily cease.

Consequently in the loss of this morphological character we have found a natural explanation for one of the much discussed special cases, viz, why the infective power of fungous germs should cease with lapse of time and with exclusive maintenance outside of the host. I will show later that as a matter of fact infections with these germs were without result, but first I will state briefly that in its composition and concentration the nutrient solution remained exactly the same during the entire period of the serial cultures; that therefore influences of nutrition and of the method of culture could not have brought about in the conidia the gradual cessation of thread germination.

The pure and satisfactory material from the sprout conidia of these smut fungi and of some others was also incidentally examined as to its possible power to induce alcoholic fermentation in nutrient solutions rich in sugar. But the forms investigated proved incapable of fermenting sugar, and could not grow at all in some of the larger masses of fluid. In these the sprouting remains nearly stationary and the germs finally die, probably from lack of sufficient access to air. From this behavior of the sprouts of the smut fungi in large masses of fluid

we can perhaps also judge of the behavior of the same germs in earth, and therefore in the soil of the field. It is scarcely to be supposed that the germs can be active in the deeper layers of the soil. It is much more probable that the condition for the vegetation of the smut fungi outside of the host plants is given only on the surface or in its vicinity, and that thence the host plants will be attacked by the smut germs.*

But, now, before I pass to the infection experiments, *i. e.*, to the production of smut diseases by infection with germs cultivated in nutrient substrata outside of the host plants, it may be judicious to state how extensive were the earlier experiments made directly and simply with the smut spores and to what results they have led.

When the smut spores dispersed in water were brought upon the surface of that part of the host in which in smutted plants the smut appears the result was purely negative; neither the penetration of the fungous germ into the plant, nor the subsequent sickening of the same, was to be observed. According to this the places where the smut shows itself in the full-grown host plants and the places where its germs penetrate into the plants could not well be the same. But neither on any other part of the full-grown plants was to be observed either the penetration of the germs or the subsequent appearance of the smut on the infected spots.

Following the earlier statements of Hoffmann it remained for J. Kühn of Halle, an eminent authority in the domain of Mycology and especially in that of smuts and smut diseases, to select young seedlings as the subjects for further experiments and observations. Kühn was the first who showed in the case of the stone smut of wheat the germ threads of the *Tilletia* in the young seedlings near the root node. Then he succeeded in doing the same thing in various forms of the genus *Ustilago*, after previous infection with the smut spores of these particular forms,—in *Ustilago maydis*, the corn smut, where three weeks after the infection he found a smut pustule in the young axis of the seedling, during the development of which the plant died; in *U. destruens*, *U. Crameri* and *U. Tulasnei*, on various kinds of millet seedlings (*Hirse-keimlingen*); and also in the dusty oat smut, *U. carbo*, and in *U. bromivora* on *Bromus secalinus*. In each case he was able to establish the existence of the mycelium of the smut fungus not only in the root node and its vicinity, but also in the first stem node and sheath-leaf node and in the internode between these and the root node. Also at the same time, in these parts, he saw distinctly the points of penetration of the fungous germs.

From his numerous experiments lasting many years, by which he

* The experiments mentioned here upon the possible fermenting power of the smut yeasts, as well as the serial cultures themselves, were conducted with the most extraordinary care. The entrance of a single other yeast germ capable of causing fermentation would of course be enough to set up fermentation in the saccharine nutrient solutions and lead the experiments to wholly erroneous results.

also unquestionably showed the smut disease in the full-grown plants after previous infection of the seedlings, Kühn reached the conclusion that the regular way for a successful infection with smut fungi was through the axis of the host plant in the first stage of germination. Besides Kühn, R. Wolff also made successful infections with smut fungi in the botanical institute of De Bary in Halle. He repeated all the earlier experiments as to the places of penetration of the fungous germ into the host plants. In experiments which he made with *Ustilago carbo*, the dusty smut of oats, and *Urocystis occulta*, the stem smut of rye, he found that the germs of these fungi could not penetrate into the full-grown parts of the host plant; that only the *sheath leaf* of the young, just germinating host plants, is susceptible and shows clearly places of penetration. In his experiments he sprayed his young plants with smut spores dispersed in water, making use of an atomizer to secure fine droplets, which alone would adhere to the surface. The plants were then kept moist under a bell glass in order to favor the germination of the smut spores on the surface and the penetration of the fungous threads. According to the conclusion which the author draws from the sum total of his experiments, the penetration of the fungous thread takes place in the young sheath-leaf of the seedling, and here only. The germs which have penetrated grow crosswise through the young leaves of the seedling till they reach their subsequent nidus of development. This permeating growth was also observed directly in *Urocystis occulta*.

Moreover in this fungus, and only in this, was observed the appearance of the smut in full-grown rye plants which were infected in their youth. To these results reached by Wolff, viz., that the sheath leaf of the young grain plant is the only place of penetration of the smut germ into the host plants, and that the germs grow crosswise through the young seedling and penetrate to the apex of growth—Kühn soon after replied with his complete array of proof, satisfactorily maintaining and additionally fortifying his earlier view. He points out that infection through the sheath leaf, which Wolff assumes to be exclusive, is uncertain, and that while in *Urocystis occulta*, according to his numerous and many-sided experiments, smutted rye can be produced by infection of the sheath leaf, this is not possible in *Ustilago carbo* on barley, in *U bromivora* on *Bromus secalinus*, and in forms in which the ovaries alone are smutted. He then sums up his experiences as follows: In all smut fungi which do not live in leaves, the result of infection through the sheath leaf is uncertain. Since these experiments by Kühn, which were made public in the Natural History Society in Halle in the beginning of 1874, no further accounts by other authors of infection experiments with grain smuts have appeared.

For my experiments, to the communication of which I now proceed, I chose the dusty smut, *Ustilago carbo*, on oats and barley, the millet smut, *U. cruenta* on *Sorghum saccharatum*, and finally the corn smut,

U. maydis on *Zea mays*. The first two smut forms will answer as types for smut fungi living solely in the grain, the last form as a type for smut forms which may appear not only in the grain but also in every part of the host plant, from the period of its earliest youth to complete development. All these forms belong to the genus *Ustilago*, and yield by the cultivation of their spores in nutrient solutions an endless sprouting of yeast conidia as experiment material.

The agriculturists, who listen to me, will perhaps ask themselves the question, Why not then make the experiments with the stone smut of wheat, the most important smut form upon the grains of this country? To this I will reply at once that stone smut was purposely neglected for reasons not far to seek. The stone smut of wheat, belonging to the smut genus *Tilletia*, is not a suitable object for experiment, first, because, as infective material, its conidia, cultivated in nutrient solutions and "reproduced mold-like in the air" can not be distributed so evenly and well upon the host plants, on account of their difficult dissemination and use in fluids, as can the the sprout conidia of the genus *Ustilago* which are developed under fluids; second, because in *Tilletia*, especially, the observation of the penetration of the germ into the host plant, and the further development of this within the host, is surrounded with the greatest difficulties on account of the extraordinary minuteness of the *Tilletia* germs and mycelia. Finally, it is yet to be added that in its appearance as a parasite, in the exclusive formation of the smut beds in the ovaries of the wheat, the stone smut agrees throughout with oat and millet smut, so that the results obtained in infection experiments with these plants will also unquestionably answer for the stone smut of wheat.

I will now first describe in detail the execution of the infection experiments and will add to this the results which were obtained in the different series of experiments with the isolated smut forms named.

In order to procure sufficient infective material, by the cultivation of smut spores in nutrient solutions, I proceeded in the following manner: Having the year before with the greatest care procured pure and ripe smut masses, I allowed single spores of *Ustilago carbo*, *U. cruenta*, and *U. maydis* to germinate in March or early in April in nutrient solutions on glass slides. After I was convinced, by exact observation, of the entire purity of the cultures and of the sprout conidia developed in them, I introduced a few of these conidia by means of a flamed needle into small, shallow flasks, with broad flat bottoms and short necks, constructed for the purpose, in which I had previously carefully sterilized thin layers of nutrient solutions. The sprout conidia so transferred which, in these flasks, were under the most favorable conditions for their increase by sprouting, exhausted the nutrient solutions in the flasks in three days' time and then accumulated on the bottom as a distinct precipitate. By samples taken out it was easily possible to convince one's self of the continued purity of the culture, because

the sprout conidia of the different kinds of smut fungi named always possess a definite and characteristic form, and intruding germs can be distinguished without difficulty. It was no trouble to keep unlimited quantities of these conidia, since a large number of these flasks for securing the material for infections were always prepared at the same time, in order subsequently to unite the sprout conidia out of these different flasks.

From the previously more fully described details as to the development of these fungi through the sprouting of their conidia, we now know that the multiplication ceases with the exhaustion of the nutrient solution, and that when this happens there immediately occurs a pushing out of the conidia into germ threads, which in turn, in the space of at most two days, lengthen out, but then cease to grow, and gradually perish. By means of these threads must the fungous germs penetrate into the host plants, when they begin to germinate upon their surface. Since the development into germ threads immediately stops the increase of conidia by sprouting, and since the germ threads, as they continue to develop, grow into the host plants, it follows that the sprout conidia must be transferred to the host plants, if the infection is to be attended with the best results, at a time when they are still sprouting and have not yet grown out into threads. The conidia which have already grown out into germ threads are very liable to injury by their transferrence to the host plants, and as soon as they have grown out are scarcely able to penetrate into the latter. The most favorable period for infection is very transitory in the rapidly growing conidia, and if it is missed, a normal success of the experiment is not to be expected. In view of this, the necessary precautions were taken to have the plants which were to be infected always ready in the various stages of development required for the individual experiments at the same time that the sprout conidia just described had reached their most favorable point of development for infection.

The transfer of the sprout conidia to the host or experiment plants was done with the help of an atomizer which Wolff had already used in his infections with smut fungi, and which I had myself formerly put to manifold uses in my mycological investigations. The sprout conidia from the different culture flasks were quickly united in one flask, in the neck of which the atomizer, cautiously tested for satisfactory performance, had been adjusted beforehand. Without the use of an atomizer it is impossible to bring the fungous germs upon the experimental host plants in the necessary degree of dispersion. Only in the tiniest drops do the sprayed fluids remain sticking to the parts of the plant upon which they have fallen; in case of larger droplets there occurs at once a union into drops which flow off, and consequently hinder the development on the plant and the penetration into it of the germs transferred with the droplet.

But it is now known that fungous germs are easily injured when taken in full vegetation and suddenly transferred from nutrient solu-

tions to pure water. They frequently die or, at all events, experience a weakening in their further development. In order to avoid these possible injuries to the sprout conidia of the smut fungi, I transferred the germs while still sprouting vigorously, from the culture flasks to the atomizer with the precaution to place in this first a diluted and sterilized nutrient solution. I knew to a certainty by previous experiments that in this the sprouting germs would not be injured, but rather would continue to sprout for a short time so far as the nutrient substances made this possible. In this way, it is true, a new source of error, the loss of time, is introduced, namely, the time in which the germs, sprayed upon the host plants by the atomizer, continue to sprout in the surrounding droplet before they grow out into germ tubes. However, without this error the experiment sometimes fails, because we can not spray into the host plant or administer to it the fungous germs in such ways as is customary in experiments with animals, but must apply them externally. The mixing of the fungous germs with the diluted nutrient solution in the atomizer can be regulated at pleasure according to the quantity of the germs. The mixture was always exactly tested before each experiment and not used until the trials each time had shown that at least thirty germs were present in the tiniest mist-like droplets.

The infection itself, to wit, the spraying with the fungous germs, was performed in shallow tin boxes made for the purpose. In this the nascent seedlings of the host plant were placed entirely uncovered, on soil from the field, and after the spraying, could be kept by means of a glass plate cover in a uniformly and suitably damp atmosphere at about 10° C. This was to hinder the evaporation of the sprayed droplets and at the same time to favor the development of the sprout conidia into threads and the penetration of these into the host plants. After 10 to 12 days the infected plants were set out in the open field, so as to make possible their full development and at the same time to give an opportunity for the development of smut in their spikes. But even with these very careful methods there were still serious obstacles to the success of the infection. The young seedlings exude, through stomata, especially at the apex of the shoot, drops of water which in running easily wash away the fungous germs which have been sprayed on, and in consequence may hinder their penetration and thus affect the result of the experiment. This and the already intimated sources of error in infections, *i. e.*, in the transference of the germs directly to the seedlings, make it probable at first sight that the infection will not succeed equally well in all the plants used for experiment, but rather that it will be successful only in a portion of these. But this indefinite per cent. of accidents is still further much increased by the circumstance that in the different forms of grain-smut the receptive stage in the seedling is so very transitory that (as later results of experiments show conclusively) only those fungous germs which penetrate into the just developed seedling above the root node, and in this

way reach the apex of growth, finally come to development in the heads of the grain; all others fail.

With this, we come to the penetration of the smut germs into the host-plants, so often vainly looked for until the investigations of Kühn and Wolff threw additional light upon the subject, and till Kühn proved the penetration into the young seedlings, especially in the vicinity of the root node. Wolff later announced and represented in his drawings the penetration exclusively into the sheath leaf. Both observers in their investigations had naturally worked only with smut spores germinating imperfectly and irregularly in water.

For my observations I first began with very young seedlings. As soon as the plumule appeared (and the roots usually preceded this by a day or two) these seedlings were laid free on the earth, sprayed with the atomizer, and then examined after several days' maintenance in suitably damp air. From all parts, from the apex to the root node, pieces of the epidermis were removed carefully and their surface examined for places of penetration. These were not to be found until the third day and were to be seen with most certainty on the fourth day; later they became gradually obscure. The spots at once attracted attention by a distinct hole in the epidermis. Beneath and inward from this hole, which was often of considerable size, extended always the intruded germ tube which had already grown crosswise through the superficial cell layers and disappeared with its apex in the deeper tissues. The influence of the nutrient material inside the cells of the host plant produced a marked effect on the germ tubes. The tubes here increased visibly in thickness and in vigorous appearance, and already in the deeper optical sections showed branches, which only very seldom appeared in germinations of the conidia in exhausted nutrient solutions.

In favorable preparations portions of the surface were found which appeared as if riddled by drill-holes and were completely permeated by the numerous ingrown germ tubes to a degree not possible to be observed, even approximately, with infective material previously employed. The more recent the places of penetration, the easier it was to see the superficial conidia in direct connection, through the epidermal opening, with the germ tube which had penetrated into the surface cells. After a time this picture lost in distinctness, in proportion as all parts of the fungous germ lying on the outside became empty and transparent and only the penetrated fungous thread bore contents. Still later the hole at the place of penetration disappeared and the germ threads in the outer cells lying near the place of penetration were transformed into delicate, empty threads, still to be recognized as fungous threads, only by the deeper union with normal portions of the tubes. I am inclined to believe that these rapid changes of the penetrated fungous threads take place because of the further growth and consequent stretching of the tissue of the seedlings, which were always infected in their earliest stages, long before they were full grown, and consequently before their

individual parts had reached full size. The fungus germs can follow this stretching of the tissue of the host plant only at their extremities, not in the remoter, older parts, which are incapable of intercalary growth, and which, consequently, being subject to strain, must be obliterated by being drawn out into threads.

Even in the next series of experiments in which older plants were infected; that is, somewhat older seedlings in which the sheath leaf was over a half inch long, but not yet broken through, the places of penetration occurred more rarely, and where they were to be seen many of the penetrated germ tubes had ceased to grow in the outer cell layers. They then exhibited an entirely different appearance, viz, a strong swelling of the membranes, which was often associated with a yellowish color. These objects had an unmistakable likeness to Wolff's drawings of the penetration spots, which the author has described as forming a cellulose sheath around the penetrating germ tubes. I have never seen such a sheath in normal cases of penetration and I consider it quite probable that Wolff only saw imperfect spots of penetration, with swollen germ tubes which he mistook for cellulose sheaths, because he confined his inflections solely to the sheath leaf in which, in somewhat older stages, the penetrated germ tube can not push in any further. (Wolff, *Brand des Getreides*, Halle, 1874.)

In order to follow up these observations I made repeated infection experiments with seedlings in which the sheath leaf was nearly full grown and was already broken through for half an inch by the following leaves. Here from the root node to the uppermost point I found no longer any normal spots of penetration. Very rarely a thread was found which had pushed through the two outer cell layers, then ceased its penetration and slowly perished with swelling of its membrane. At the same time there lay upon the surface hundreds of germinated conidia which could no longer penetrate, because the epidermis, fully formed in the meantime, was no longer permeable. The seedlings, therefore, in this stage of development already behaved toward the fungous germs exactly as do all parts of fully developed plants, into which, as is well known, the threads can not penetrate and in which they can not grow further.

Up to this point of the investigation, therefore, my observations confirmed, with some additions and amplifications, the earlier results of Kühn and some statements of Wolff. Nevertheless, it would appear to me that they only partially exhaust the question as to the place of penetration of the smut germ into the host plant, and that even the new proof material which supported the old idea hitherto generally accepted, that the smut germs must penetrate into the young seedlings in order later to produce smut in the full grown plants, is still insufficient and can not well be regarded as definitely concluding the investigation. For why should the penetration occur only in the young seedling which possesses no other disposition for it except the immaturity

of its tissues, which allows the penetration of the germ? Do not all incipient tissues of the growing tip of full-grown plants likewise exhibit this immature condition?

With our ordinary cereals further experiments did not, indeed, appear to be practicable on account of their small size. The growing tips of oats, barley, wheat, etc., are too small; it is here scarcely possible to bring the fungous germ into the still closed parts of the bud; the young ovaries are also too minute to work on with sufficient clearness of view. But I will nevertheless add that the penetration of fungous germs which I had here introduced into the heart of the growing point by means of the long drawn out point of a spraying flask, was established by direct observation and the threads of the penetrated germs could be seen in the leaves.

But the long series of experiments which I conducted with the larger cereals, corn and sorghum (*Hirse*), proved this much more convincingly. Here the tip is more open. The unexpanded leaves of the bud, folded one within another, open in the form of a large cornet into which we can spray with the syringe flask unlimited quantities of the nutrient solutions containing sprout conidia. These soak down deep between the closed leaves, and in corn, can even reach the growing tip itself with its young staminate panicle, in case the latter, in a somewhat advanced stage of development, has already pushed upward far enough in the bud. Furthermore, in corn the large adventive incipient roots on the lower part of the axis, and the pistillate spikes, particularly, which appear later upon the fully developed axis as sprouts in the leaf axils, offer excellent places of attack. Of course these experiments had to be made on large plants, or, in case of the infection of pistillate spikes, on nearly full grown ones, *in the open air*, where any other protection than a temporary covering with large straw mats was no longer possible.

In order, first, to consider the experiments with sorghum and *Ustilago cruenta*, its associated smut, I will add that I have infected in the heart more than 600 plants from 1 to 3 feet high, by simple injection of the fluid containing the sprout conidia. After four days the further developed portions of the growing point, in so far as they had come into direct contact with the infective fluid, appeared somewhat yellow. Upon superficial sections, the picture of the penetration of the fungous germs was a very clear one. The whole surface was covered with holes, from which big and luxuriant tubes extended into the inner parts of the young leaves, while through their influence was brought about obviously a faint yellowing, and later a more or less distinct wrinkling and shriveling of the attacked leaves. In thin cross-sections were to be found dozens of penetration spots cut through accidentally, while the fungous tubes grew through the entire tissue of the young leaves. That in this case only the young leaves were accessible to the fungous germs was shown on the older portions of the other leaves, which, though

richly covered with germinating conidia, did not show a single penetration spot.

The experiments with corn and corn smut were carried on still more comprehensively. Infections in the heart were first made on young plants about 6 inches high and showing an open apex, up to those of more than 2 feet in height. The appearances of penetrations were uniformly observed in all parts of the young leaves and young axes, in just the same manner except that on account of the size of the corn plants they were yet more distinct than in the sorghum. All the young leaves of the bud, and the still short and unexpanded parts of the axis lying between, were susceptible to penetration, as well as the tips of the axes with the staminate panicles, when the latter were reached by the spraying of the infective fluid. The penetrations also ceased here only when all parts of the bud passed from immaturity to full development. Concerning the adventive roots, and also the side sprouts of the pistillate spikes, which appear later and were infected in the bud, I can assert exactly the same thing as for the buds of the main axis; and, finally, I will state merely for sake of completeness, that also the scattering young hairs on the leaves, which are incipient in the very young leaves, are readily attacked by the fungous germs. Penetration spots were to be seen on these with especial distinctness.

After all possible places of attack by the smut germs have been discovered, there now remains to be added the results which were obtained in the subsequent production of smut upon the proper host plants with the specified smut fungi, by means of the various sorts of infection. This is done for the purpose of arriving at such conclusions as may be drawn with scientific authority in regard to the susceptibility of the host plants used in our experiments to smut diseases at different ages and stages of development, and on the appearance and spread of such diseases.

A. I begin with *Ustilago carbo*, the notorious dusty smut which destroys the fruit of oats, barley, wheat, etc. The smut spores germinate easily and produce sprout conidia in endless generations in nutrient solutions. In mass, the sprout conidia have a hyaline appearance. Their membranes become a little slimy on the outside, so that the germs can not lie together closely, but often form loosely connected heaps, which can again be easily dispersed in fluids.

The infections by dusty smut (*Flugbrand*) were carried on with barley and oats at the same time, and altogether considerably over one hundred series of experiments were made. In order to exclude sources of error, sowings of the uninfected grains were made for comparison, concerning which I will state, in brief, that they brought forth sound culms and fruit, only showing one smutty plant in two cases.

I. For the first series of experiments the grains were chosen particularly in the earliest stage of germination, where the rootlets had already come forth and the plumule was just visible. The tiny plants were placed upon the earth uncovered and were sprinkled all over with sprout

conidia from the atomizer. The culture remained about ten days in a room at 10° C., under cover in the tin boxes previously described and then the plants were set out in the field.

In ten experiments with oats, always with a sowing of 100 grains, the result was on an average 17 to 20 per cent. of smutty panicles. The infected barley remained entirely sound.

II. In the following series of experiments the grains which barely showed rootlets were placed on the earth and so covered with a thin layer of soil, at most ¼cm. thick, that only the emerging points of the seedlings were exposed and were infected by means of the atomizer, consequently the infection only reached the sheath leaf. The shoots were infected in the youngest stage when they had pushed out of the earth about ¼cm.

In seven experiments with oats, each of 100 grains, the result was not more than 5 per cent. of smutty plants. The barley remained entirely sound.

III. The infection was made as in I on uncovered plants, the shoots of which were about 1½–2^{cm} long, but did not yet show any opened sheath leaf.

Here in eight experiments with oats the result fell back to 2 per cent. of smutty plants; barley sound.

IV. Infection as in II, the sheath leaf only infected, the remaining parts of the seedlings covered with soil, but the shoot of the same length as in III.

In three experiments with oats there was 1 per cent. of smutty plants; in two experiments none were obtained; barley sound.

V. Infection of uncovered seedlings with sheath leaf already pushed through.

In two experiments with oats the result was 1 per cent. of smutty plants, in two others, none; barley sound.

VI. Experiments with infected soil in which the unsprouted grains were sown.

In five experiments with oats the result amounted 4 to 5 per cent. of smutty plants; barley sound.

VII. Experiments with an abundantly infected mixture of soil and fresh horse dung, in which the unsprouted grains were sowed.

Here in three experiments with oats the result rose to 40 to 46 per cent.; in three additional experiments, which were not conducted in a cool room, there was 27 to 30 per cent. of smut; barley again entirely sound.

VIII. Experiments with conidia, which had been cultivated ten months, generation after generation, in nutrient solutions, and which ceased to grow out into threads after the exhaustion of the solutions, infection of young seedlings lying uncovered on the earth in first stage of germination, as in I.

The result was negative. In two series of experiments there was in

one case 1 per cent., in the other 2 per cent. of smutty plants; in two additional series no smutty plants; barley sound.

IX. Experiments with larger plants by external infection and by infection in the heart of the growing tip, were wholly without result.

The final result of the experiments with oats may be summed up as follows: The infection most productive of results is upon the barely germinating young seedlings, just as it was previously stated by Kühn. The exclusive infection of the sheath leaf is fruitful, as a rule, only in the youngest stages of the same. The infection is without result as soon as the inner leaves have pushed through the sheath leaf more than 1cm.; from this point on the plants are proof against the fungous germ. By the use of nutrient substrata for the conidia sproutings, consequently by means of earth treated to fresh horse dung, the infection of the young seedlings will be greatly increased and the spread of the smut very materially promoted,* corresponding to the experience of husbandmen in the use of fresh dung in the field. Smut germs, which have lived too long and too exclusively outside of the host plant and multiplied in the form of sprout conidia, lose their infective power conjointly with the ability to throw out germ tubes.

But how are the negative results of the experiments to be interpreted? *First*, how is it to be explained that even in the most favorable cases only a large per cent. of the experimental plants become smutty and not all which were infected? And, *second*, whence comes it that in all experiments with barley in not one single case did a plant become smutty?

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* The influence of fresh dung on the production of grain smuts diminishes quickly with the age of the dung, because the conidia germinated in it perish, and in old rotten dung the smut spores develop imperfectly or not at all. The less wet the dung the more slowly decay takes place, and the longer the smut germs can maintain themselves in it.

In the dung of horses, and of swine also, are to be found many oat and barley grains which have not been digested and which subsequently germinate in the dung. Many times by the hundred in root fields I have come across such germinated barley grains, accidentally transported into the field with the fresh swine dung, and have found that for the most part they bring forth smutty spikes. This bears most striking witness to the effect of *fresh* dung in the spreading of smut and in the appearance of smut in *freshly manured* fields. In isolated cases, in small fields, I have gathered the smutted spikes in thick bundles, and have found that out of 100 barley plants were to be found only 10 to 15 sound spikes. It need not be said that in these cases I have each time inquired very exactly and particularly concerning the way of manuring.